AQUA-FAANG - Standard Operating Protocol - OmniATAC protocol in carp embryos

Overview

This protocol describes a method for transposition and ATAC-seq library preparation using isolated fresh or frozen nuclei from carp embryos. This protocol is based on Buenrostro et al., 2015 (1) and the OmniATAC protocol from Buenrostro et al., 2017 (2). The resulting version adjusted by the Macqueen laboratory at the Roslin Institute (3) is shown below. Steps modified by the Mueller lab (University of Birmingham) for ATAC in carp embryo samples have been highlighted in green.

Nuclear extraction can be achieved following the corresponding University of Birmingham Standard Operating Protocol in the FAANG portal (4).

1. Transposase reaction and clean-up

1.1. Prepare the following transposase reaction mix:

Reagent	Volume per sample (µl)
2x Tagment DNA (TD) buffer	25
Transposase	1.25
PBS	16.5
Tween-20 (10%)	0.5
Nuclease-free H_2O	6.75
Total	**50**

*Note: The Mueller lab transposase reaction mix does not include digitonin and therefore the volume of water has been readjusted to account for this.

1.2. If using freshly extracted nuclei resuspend the pellet in 50µl of transposase reaction mix by pipetting up and down 6 times.

1.2.1. If using frozen nuclei, thaw samples on ice, spin down at 500g for 5 mins, 4°C and carefully remove (all) supernatant before resuspending in transposase reaction mix.

1.3. Incubate the reaction at 37°C for 30 minutes in a thermomixer at 900RPM.

1.4. Stop the reaction and purify the resulting DNA fragments with MinElute PCR purification kit.

1.5. Elute DNA in 21 µl EB (Present in MinElute PCR purification kit)
1.6. Eluted DNA can be stored at -20°C until ready to amplify.

2. Library amplification

Overview: This section describes the process used to generate amplified libraries starting from eluted transposed DNA. To reduce GC and size bias in PCR, the appropriate number of PCR cycles is determined using qPCR to see when to stop the amplification prior to saturation.

Reagents:

- 96 well PCR plate or PCR tube strip, depending on number of samples.
- PCR cap strips.
- qPCR plate.
- qPCR compatible cap strips.
- IDT® for Illumina® Nextera™ DNA UD Indexes.
- NEBNext® Ultra™ II Q5® Master Mix.
- 10.000x SYBR™Green.
- Nuclease free water.

Preparation:

- Make sure PCR and qPCR equipment will be available when needed.
- Thaw transposed DNA, indexes, Master Mix, and SYBR™Green.
- Invert the tubes to mix and spin down.

2.1. Save the following program (ATAC-PRE) on a thermal cycler with a heated lid:

Temperature (°C)	Time
Initial 5 min extension	
72	5min
98	30sec
Repeat 4 times (5 cycles total)	
98	10sec
63	30sec
72	1min
4	Hold forever

2.2. Set up the following PCR reaction:

Reagent	Volume per sample (µl)
IDT® for Illumina® Nextera™ DNA UD Indexes	5
NEBNext® Ultra™ II Q5® Master Mix	25
Sample (Transposed DNA)	20
Total	**50**

2.3. Mix reagents, seal plate and centrifuge at 280RCF, 20°C, 1min

2.4. Place the plate on the pre-programmed thermal cycler and run ATAC-PRE

2.5. Using 5 µl (10%) of the pre-amplified mixture from step 2.4, run the following qPCR reaction to determine the number of additional cycles needed*:

*This plate can be prepared at the same time as the PCR-reaction in step 1. Dilute 10.000x SYBR™Green to 25x in NF-water (1:399)

Reagent	Volume per sample (µl)
Nuclease free water	3.76
IDT® for Illumina® Nextera™ DNA UD Indexes (same ones as for original PCR reaction)	1
NEBNext® Ultra™ II Q5® Master Mix	5
Pre-amplified sample from step 2.4	5
Total	**15**

2.6. When ATAC-PRE has finished, remove plate/tubes from the thermal cycler and add 5µl of each pre-amplified library to the qPCR reaction prepared in step 2.5.

2.7. Mix reagents, seal plate and centrifuge at 280RCF, 20°C, 1min.

2.8. Cycle in a qPCR thermal cycler as follows:

Temperature (°C)	Time
98	30sec
Repeat 19 times (20 cycles total)	
98	10sec
63	30sec
72	1min
4	Hold forever

2.9. Manually assess the amplification profiles and determine the required number of additional cycles to amplify. The number of cycles should be equal to ¼ of max fluorescence (Fig. 1). This is to ensure that the amplification is stopped prior to saturation to avoid PCR bias.

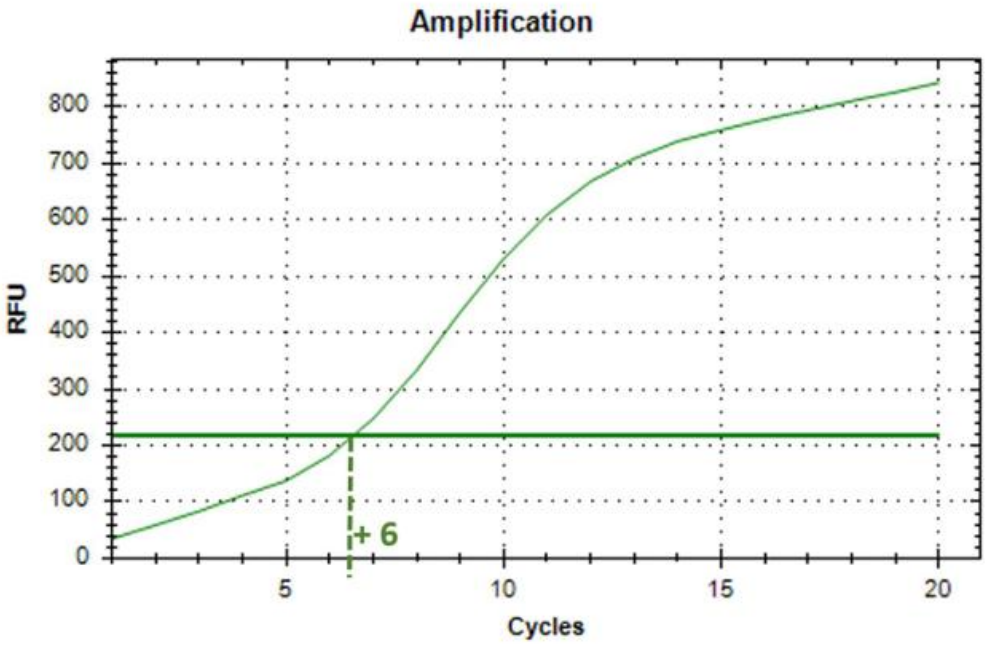

Figure 1. qPCR amplification plot showing ¼ of max fluorescence = 6 additional PCR cycles

2.10. Save the following program (ATAC-AMP) on a thermal cycler with a heated lid:

Temperature (°C)	Time
98	30sec
Number of cycles determined in step 2.9	
98	10sec
63	30sec
72	1min
4	Hold forever

Choose the preheat lid option and set it to 100°C.

2.11. Seal plate and centrifuge at 280RCF, 20°C, 1min.

2.12. Place the plate on the pre-programmed thermal cycler and run ATAC AMP.

2.13. Optional stop point – Continue directly to library clean-up or store the amplified libraries at 4°C for up to two days.

3. Library clean-up and assessment

Overview: This section describes the methodology followed to perform double sided bead purification on the previously amplified samples, in order to get rid of big (>670bp) and small fragments (<100bp). This is followed by assessment of libraries using a HS D5000 screentape.

Reagents and consumables:

- Magnet (for Eppendorf tubes or 96 well plate, depending on number of samples)
- Freshly prepared 80% ethanol.
- Room temperature Ampure XP beads.
- LoBind Eppendorf tubes or Sarstedt Microtest 96 well plate, conical bottom.
- Room temperature resuspension buffer (TET buffer):
    - Tris-HCl pH 8.0 (1M): 10mM final concentration. 16.5µl for 100 samples.
    - EDTA (0.5M): 1mM final concentration. 3.3µl for 100 samples.
    - Tween-20 (10%): 0.05% final concentration. 8.25 µl for 100 samples.
    - Nuclease free water: 1621.95µl for 100 samples.

3.1. Thoroughly resuspend Ampure XP beads by vortexing for at least 1min.
3.2. Add 0.53x volume (23.9 µl if you have 45 µl) of beads to sample. This will preferentially bind to longer DNA fragments).
3.3. Mix well by pipetting but be gentle to avoid bubbles.
3.4. Incubate at room temperature for 10min, while mixing gently every 5min.
3.5. Touch spin to collect liquid, then place tube on magnetic rack and allow to stand for 5min (until supernatant is clear of beads).
3.6. Transfer the supernatant to a new tube and another 1.3x original volume (58.5 µl if you had 45 µl) Ampure beads to the supernatant.
3.7. Mix well by pipetting but be gentle to avoid bubbles.
3.8. Incubate at room temperature for 15 min while mixing gently every 5 minutes.
3.9. Place on magnetic rack and let it stand for 5 min (until the supernatant is clear of beads). Remove and discard supernatant.
3.10. While the tubes are still on the magnet, and without disturbing the pellet, wash beads with 100µl of freshly prepared 80% ethanol, remove ethanol and discard. Repeat once.
3.11. Remove the tubes from the magnet, touch spin the samples, place back on the magnet and carefully remove the last traces of ethanol.
3.12. Remove samples from the magnet and allow the tubes to air dry between 30 sec-2min. Do not wait until you see small cracks in the beads!
3.13. Add 16.5µl TET Buffer. Resuspend beads by pipetting.
3.14. Rehydrate at room temperature for a minimum of 2 minutes.

3.15. Place on magnetic rack and let it stand for 5 min (until the supernatant is clear of beads).

3.16. Transfer the supernatant (eluted DNA) to a LoBind Eppendorf tube or 96 well plate.

3.17. Add 1µl of library to 3µl of nuclease-free water (1:4 dilution)

3.18. Use 1µl of diluted library to measure DNA library concentration with Qubit High Sensitivity Kit.

3.19. Use 1µl of diluted library to validate DNA fragment size distribution with Tapestation using a HS D5000 screen tape. The DNA fragment size distribution should to some extent follow a nucleosome pattern with the most prominent peak being at about 200bp. (Figure 2.)

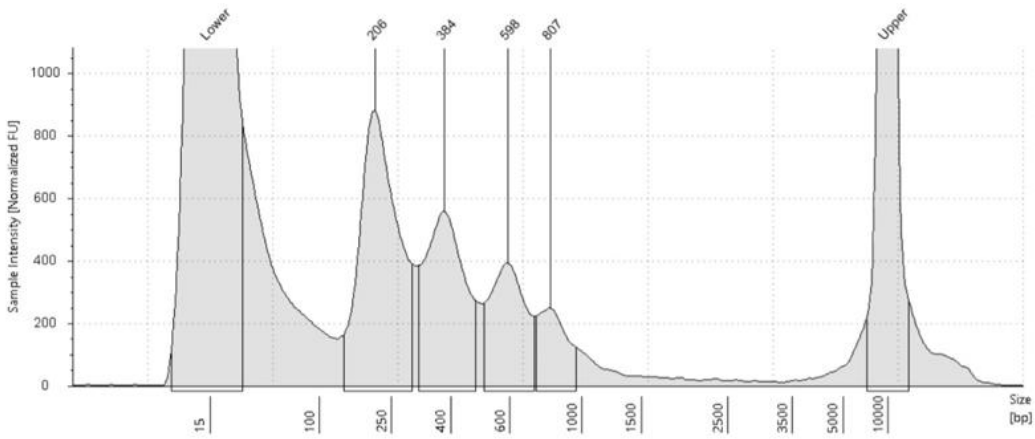

Figure 2. Example of a Tapestation profile after library preparation and clean up. This shows a very well represented nucleosome pattern with the most prominent peak at ~200bp.

Bibliography

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